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TECHNICAL MANUSCRIPT 615

CELL-SURFACE ANTIGEN INDUCED BY
VENEZUELAN EQUINE ENCEPHALOMYELITIS VIRUS

Nicholas Hahon

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DEPARTMENT OF THE ARMY
Fort Detrick
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TECHNICAL MANUSCRIPT 615

CELL-SURFACE ANTIGEN INDUCED BY
VENEZUELAN EQUINE ENCEPHALOMYELITIS VIRUS

Nicholas Hahon

Experimental Aerobiology Division
AEROBIOLOGY & EVALUATION LABORATORIES

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CELL-SURFACE ANTIGEN INDUCED BY
VENEZUELAN EQUINE ENCEPHALOMYELITIS VIRUS*

ABSTRACT

By immunofluorescence staining, a specific surface antigen induced by VEE virus was detected on L-929 cells. Induction of the antigen was dependent on viable virions; it was not, however, the consequence of viral protein coats deposited on cells. The formation of surface antigen was independent of viral RNA synthesis. It is suggested that the induction of cell-surface antigen is coded by an early function of the viral genome.

DNA virus-specific surface or membrane antigens induced on established cell cultures or on tumor cells by oncogenic viruses such as SV40, polyoma, Epstein-Barr (EB), Shope papilloma, herpesvirus of Marek's disease,¹⁻⁷ and by non-oncogenic agents, i.e., some poxviruses of the vaccinia group^{8,9} have been demonstrated by the immunofluorescent technique. Other than the detection of a new cell-surface antigen in Moloney virus - induced lymphomas and in cell cultures,^{10,11} comparable findings have not been reported with other RNA oncogenic or non-oncogenic viruses. This report describes the induction of a specific cell-surface antigen by the RNA non-oncogenic Venezuelan equine encephalomyelitis (VEE) virus.

A stock pool of the Trinidad donkey brain strain of VEE virus was prepared from infected McCoy cell monolayers;¹² its titer was 8×10^6 cell-infecting units (CIU) per ml when assayed by the immunofluorescent cell counting technique.¹² Monolayers of L-929 (mouse fibroblast) cells were grown and maintained in medium 199 with 5% fetal calf serum, 50 μ g streptomycin, and 75 μ g kanamycin per ml. The procedure for infecting confluent cell monolayers grown on 15-mm-diameter cover slips is described in detail elsewhere.¹² To demonstrate cell-surface antigen, infected cell monolayers were incubated at 35 C for 7 to 8 hours, rinsed with phosphate-buffered saline (PBS), pH 7.1, and, without fixation, stained by the direct immunofluorescent technique. Surface antigen was unstainable by acetone fixation of cells for 5 minutes at room temperature. The preparation of monkey antiviral serum, conjugation of the globulin fraction with fluorescein isothiocyanate, and procedures for staining and counting infected cells have been described previously.¹²

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Surface antigen appeared on VEE virus - infected cells as bright rings of fluorescence (Fig. 1); uninfected cells did not fluoresce. The specificity of the staining was confirmed by the absence of surface fluorescence on cells infected with VEE virus and stained with variola, chikungunya, or yellow fever fluorescein-conjugated antiviral serum. Similar results were obtained when cell monolayers were infected with any of these three viruses and stained with conjugated VEE antiserum. Induction of cell-surface antigen was not limited to virus grown in cell cultures, the Trinidad strain, or the L-929 cell line; the phenomenon was elicited by both infective mouse brain and chick embryo preparations, by two different attenuated VEE virus strains,^{13,14} and on guinea pig lung, McCoy, and baby hamster kidney (BHK21/C13) cell lines. Induction of cell-surface antigen by VEE virus is dependent on the presence of infectious virus particles. Virus inactivated by either ultraviolet irradiation or heat (56 C, 16 hours) failed to induce the formation of cell-surface antigen. Furthermore, the number of cells showing surface antigen was reduced approximately 93% when virus was mixed with antiviral serum before adding it to cell monolayers.

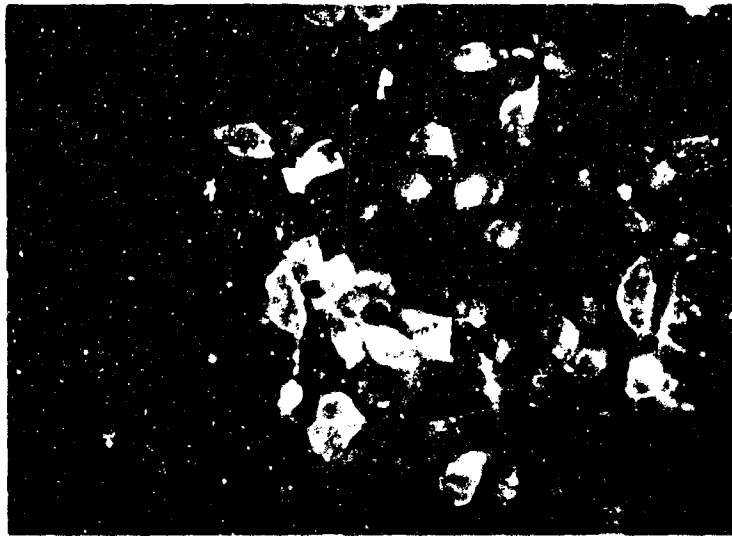


FIGURE 1. Immunofluorescence on the Surface of L-929 Cells Infected with VEE Virus. Eight hours after infection, cells were stained, without fixation, with labeled anti-VEE monkey globulin. 126X.

Additional evidence that the manifestation of cell-surface antigen is associated with the presence of infectious virus particles is based on the linear relationship between relative virus concentration and the number of cells showing surface antigen (Fig. 2). This finding suggests that each cell exhibiting fluorescent surface antigen results from infection by a single infective virion or aggregate not divisible by dilution. The dependency of surface antigen induction on viable virus is consistent with observations on the direct association between infection and/or production of EB virus and cell membrane antigen.¹⁻⁷

To compare the rate of appearance of fluorescent surface antigen on cells with that of cytoplasmic viral antigens, cell monolayers were infected with approximately 10^3 CIU of virus and, 1 hour after virus attachment, a 1/10 dilution of antiviral serum was added to each cell monolayer to prevent a secondary cycle of infection by extracellular virus. During incubation at 35 C for 20 hours, representative cell monolayers, at 4-hour intervals, were either washed with PBS or fixed with acetone and then stained. Fluorescent cell-surface antigen could be visualized 4 hours after infection and preceded the appearance of cytoplasmic viral antigen by 4 hours (Fig. 3). The number of cells showing surface antigen was maximal between 8 and 12 hours; the number was equivalent to those with cytoplasmic fluorescence at 12 hours. In view of the quantitative relationship demonstrated earlier (Fig. 2), it is feasible to assay for VEE virus infectivity by counting cells with fluorescent surface antigen as well as assessing the neutralizing content of serum. The latter prospect is based on the observation cited earlier wherein a reduction in the number of cells with surface antigen resulted when virus inoculum was first mixed with antiviral serum. The assay of Moloney leukemia virus based on cell membrane fluorescence has been found applicable for estimating the antiviral activity of serum.^{13,14}

The possibility that surface antigen may be derived from the outer protein layers of penetrated virions deposited on cell surfaces was examined by incorporating inhibitors of protein synthesis, puromycin (50 μ g/ml) or cycloheximide (100 μ g/ml) in maintenance medium, which was then added to cell monolayers immediately after virus attachment. Cell monolayers to which only maintenance medium was added showed surface antigen at 8 hours or cytoplasmic viral antigens at 20 hours when appropriately fixed at these time intervals; however, neither surface nor cytoplasmic antigens could be demonstrated in cell cultures that had been treated with inhibitors of protein synthesis. The introduction of infectious viral RNA onto cell monolayers resulted in the formation of cytoplasmic viral antigens as well as cell-surface antigen. This constitutes additional evidence that cell-surface antigens are not deposits of viral protein coats.

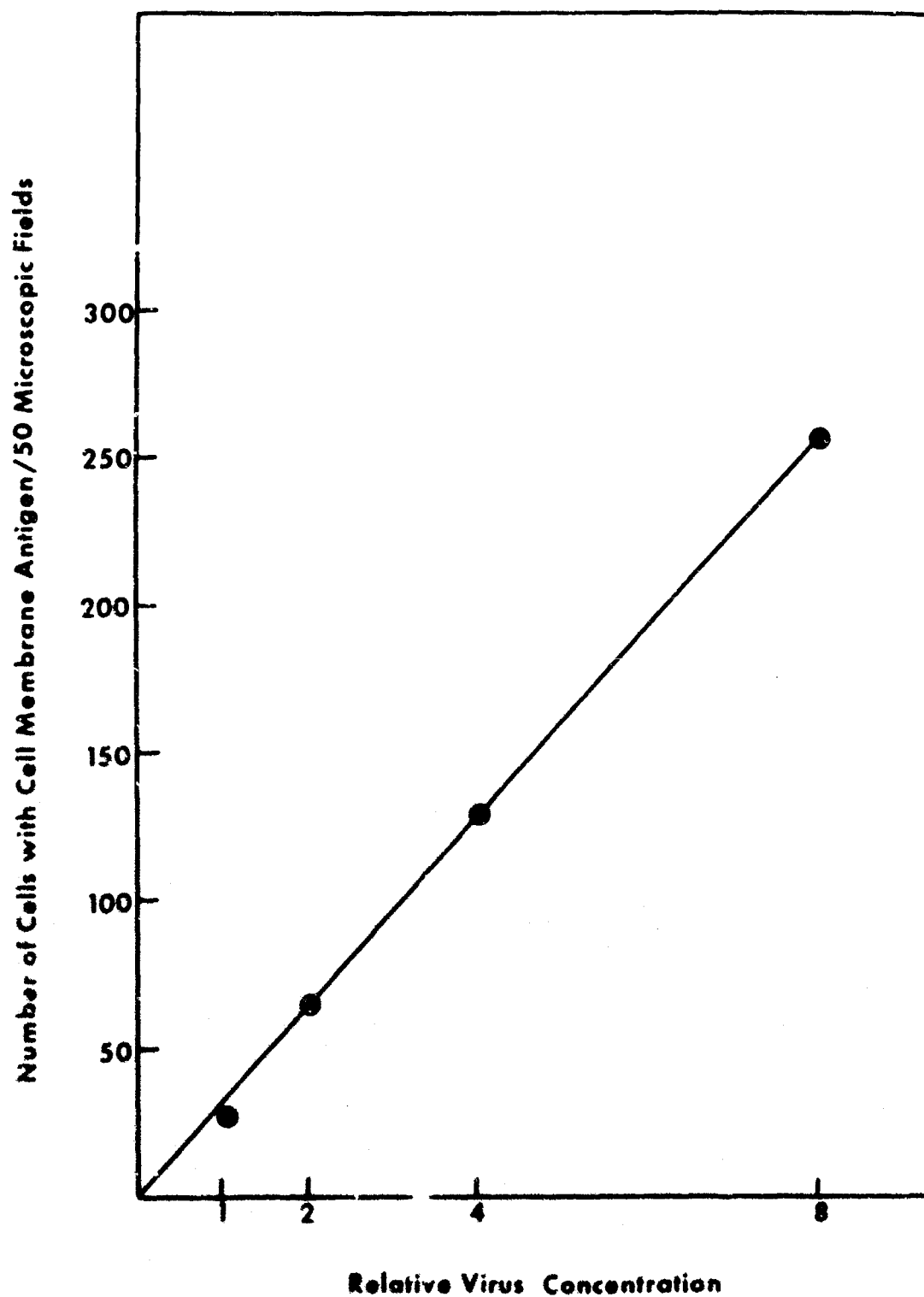


FIGURE 2. Linear Function Between Relative Concentration of VEE Virus and the Number of L-929 Cells Showing Immunofluorescent Surface Antigen.

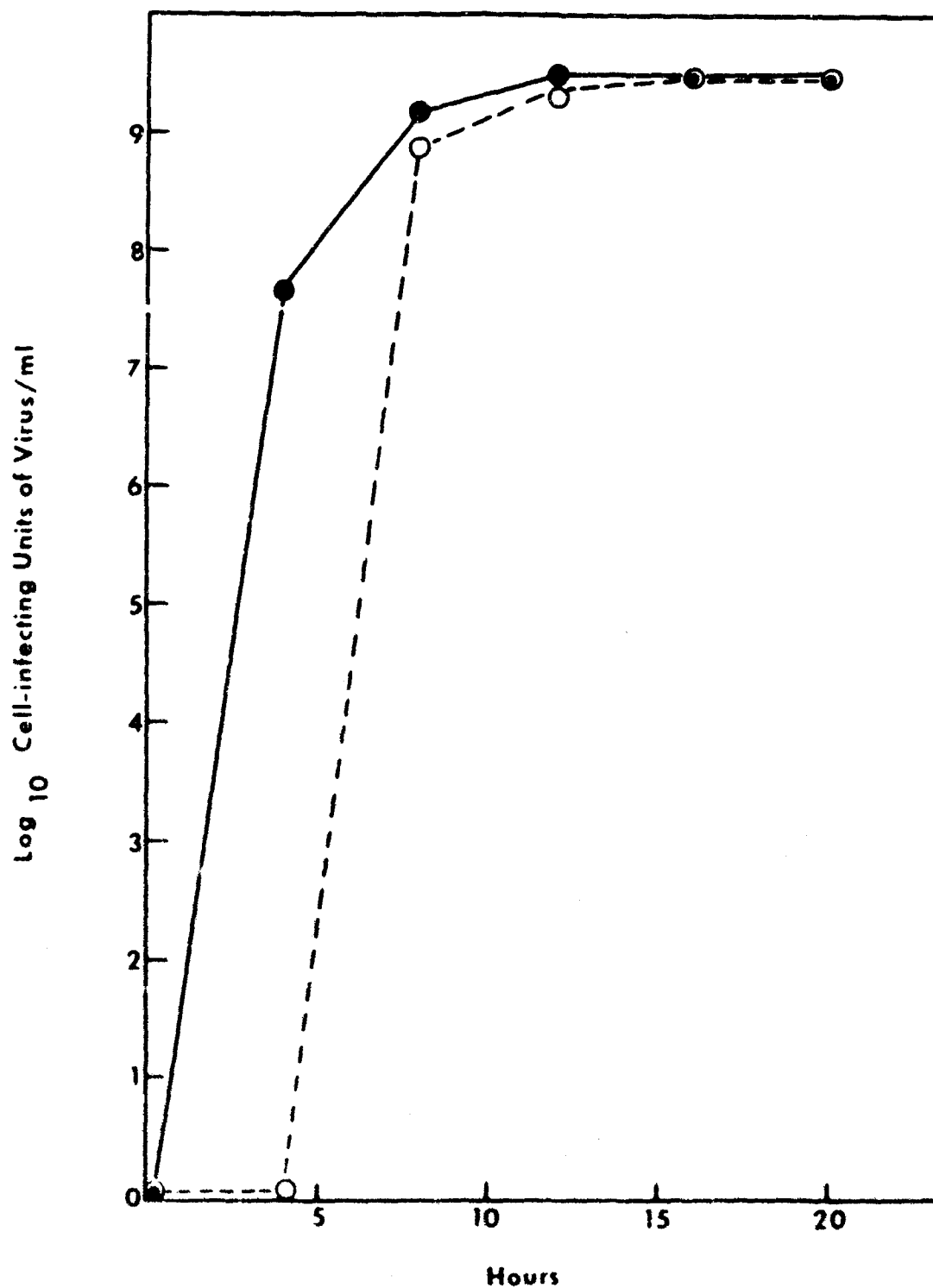


FIGURE 3. Rate of Appearance of Immunofluorescent Surface (●) and Cytoplasmic (○) Antigens in L-929 Cells Infected with VEE Virus. Cell-infecting units of virus were calculated by averaging the number of fluorescent cells per microscopic field, multiplied by the number of fields per cover slip, the reciprocal of the dilution of virus inoculum, and a volume factor for conversion to milliliters.¹²

That the addition of cytosine arabinoside to HeLa cell cultures infected with vaccinia virus suppresses the formation of cytoplasmic viral antigens but does not impair the synthesis of surface antigen is indicative of the latter's independence of viral DNA replication.^{8,9} To determine whether the production of surface antigen induced by VEE virus is dependent on the replication of viral RNA, cell monolayers were treated for 24 hours with interferon, an inhibitor of viral RNA synthesis,^{15,16} before they were infected with virus. Results (Table 1) show that the formation of cell surface antigen was unaffected by interferon but the number of cells with cytoplasmic antigen, an indicator of viral replication,¹² was reduced by approximately 93%. These data suggest that the specific surface antigen is a newly synthesized protein coded by an early function of the viral genome, a fraction thereof, or a component of the cell membrane modified by virus infection. That cell membranes possess protein-synthesizing mechanisms has been established by the recent demonstration that isolated membranes of animal cells actively participate in protein synthesis.¹⁷

TABLE 1. EFFECT OF INTERFERON ON FORMATION OF VEE VIRUS ANTIGENS IN L-929 CELLS^{a/}

| Treatment | Number of Immunofluorescent Cells/50 Microscopic Fields | |
|------------|---|---------------------|
| | Surface Antigen | Cytoplasmic Antigen |
| Interferon | 218 | 13 |
| Control | 215 | 198 |

- a. Interferon was prepared in L cells with VEE virus as the inducer and assayed by the immunofluorescent cell reduction technique.¹⁸ Cell monolayers were treated for 24 hours at 35 C with 660 units of interferon or maintenance medium (control) before the addition of virus inoculum. They were then incubated at 35 C for 8 hours and stained without prior fixation to detect surface antigen or incubated at 35 C for 20 hours and fixed with acetone before staining to detect cytoplasmic viral antigen.

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